P NT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
SCB 527 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 01044	09/02/2000	12/02/1999
Applicant		
CHIESI FARMACEUTICI S.P.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/01044

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/785 A61P11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PALMBLAD, MARIE ET AL.: "BIOPHYSICAL ACTIVITY OF AN ARTIFICIAL SURFACTANT CONTAINING AN ANALOGUE OF SURFACTANT PROTEIN (SP)-C AND NATIVE SP-B" BIOCHEM J (1999) 339(2) 381-386, April 1999 (1999-04), XP002139844 the whole document	1-16

X	WO 91 18015 A (CALIFORNIA BIOTECHNOLOGY INC) 28 November 1991 (1991-11-28)	1,10-16
Y	claims 5,8	7

X	EP 0 733 645 A (TOKYO TANABE CO) 25 September 1996 (1996-09-25)	1,10-16
Y	abstract; claims	7

A	EP 0 368 823 A (KABIGEN AB) 16 May 1990 (1990-05-16)	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2000	29/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/01044

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	TAKEI, TSUNETOMO ET AL.: "THE SURFACE PROPERTIES OF CHEMICALLY SYNTHESIZED PEPTIDES ANALOGOUS TO HUMAN PULMONARY SURFACTANT PROTEIN SP-C" BIOL PHARM BULL (1996) 19(10) 1247-1253, XP002139845 -----	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC[redacted] 00/01044

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9118015 A	28-11-1991	US 5104853 A	14-04-1992
		CA 2083177 A	18-11-1991
		EP 0538273 A	28-04-1993
		JP 5509301 T	22-12-1993
		US 5385840 A	31-01-1995
EP 0733645 A	25-09-1996	AU 682738 B	16-10-1997
		AU 1199295 A	27-06-1995
		BG 100554 A	31-12-1996
		FI 962355 A	06-06-1996
		NO 962403 A	07-06-1996
		SK 71496 A	06-11-1996
		US 5827825 A	27-10-1998
		CA 2178345 A	15-06-1995
		CN 1136813 A	27-11-1996
		CZ 9601623 A	16-10-1996
		HU 74880 A,B	28-02-1997
		WO 9515980 A	15-06-1995
		NZ 277095 A	22-09-1997
		PL 314872 A	30-09-1996
EP 0368823 A	16-05-1990	AT 84799 T	15-02-1993
		AU 623180 B	07-05-1992
		AU 4299689 A	26-04-1990
		CA 2000893 A	18-04-1990
		DE 68904530 D	04-03-1993
		DE 68904530 T	08-07-1999
		JP 2145599 A	05-06-1990
		JP 2963923 B	18-10-1999
		SE 8803713 A	18-10-1988
		US 5455227 A	03-10-1995
		US 5223481 A	29-06-1993

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 August 2000 (24.08.00)	
International application No. PCT/EP00/01044	**Applicant's or agent's file reference** SCB 527 PCT
International filing date (day/month/year) 09 February 2000 (09.02.00)	**Priority date** (day/month/year) 12 February 1999 (12.02.99)
Applicant CURSTEDT, Tore et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 July 2000 (27.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

CLAIMS

1. SP-C analogues having general formula (I), according to one-letter amino acid code:

$$F_eG_fIPZZPVHLKR(X_aB)(X_bB)_n(X_cB)_mX_dGALLMGL \quad (I)$$

wherein:

X is an amino acid selected from the group consisting of V, I, L, Nle (norleucine);

B is an amino acid selected from the group consisting of ornithine, K, I, W, F, Y, Q, N;

Z is an amino acid selected from the group consisting of S, C, F where Ser or Cys residues are optionally linked via ester or thio-ester bonds with acyl group containing 12-22 carbon atoms.

a is an integer from 1 to 19;

b is an integer from 1 to 19;

c is an integer from 1 to 21;

d is an integer from 0 to 20;

e is 0 or 1;

f is 0 or 1;

n is 0 or 1;

m is 0 or 1,

with the following conditions:

- $n + m > 0$;
- $f \geq e$;
- $(X_aB)(X_bB)_n(X_cB)_mX_d$ is a sequence having a maximum of 22 amino acids, preferably from 10 to 22 amino acids.

2. SP-C analogues according to claim 1, having formula (Ia):

(Ia) $FGIPSSPVHLKRX_4BX_4BX_4BXGALLMGL$

3. SP-C analogues according to claim 1, having formula (Ib):

(Ib) FGIPSSPVHLKRX$_5$BX$_5$BX$_4$GALLMGL

4. SP-C analogues according to claim 1, having formula (Ic)

(Ic) FGIPSSPVHLKRX$_4$BX$_{11}$GALLMGL

5. SP-C analogues according to claim 1, having formula (Id)

(Id) FGIPSSPVHLKRX$_8$BX$_7$GALLMGL

6. SP-C analogues according to claim 1, having formula (Ie)

(Ie) FGIPSSPVHLKRX$_{11}$BX$_4$GALLMGL

7. SP-C analogues according to claims 1-6, in which Ser residues are acylated preferably with palmitoyl groups.

8. SP-C analogues according to claims 1-7, in which B is Lysine or Phenylalanine and X is Leucine, Isoleucine or Norleucine.

9. SP-C analogues according to claim 8, selected from the group consisting of:

SP-C (LKS) FGIPSSPVHLKRLLILKLLLLKILLLKLGALLMGL

SP-C (LKS)$_1$ FGIPSSPVHLKRLLILLKLLLLIKLLILGALLMGL

SP-C (LKS)$_2$ FGIPSSPVHLKRLLILKLLLLLILLLILGALLMGL

SP-C (LKS)$_3$ FGIPSSPVHLKRLLILLLLLKLILLLILGALLMGL

SP-C (LKS)$_4$ FGIPSSPVHLKRLLILLLLLLLIKLLILGALLMGL

SP-C (LFS) FGIPSSPVHLKRLLILFLLLLFILLLFLGALLMGL

10. A synthetic surfactant comprising at least one SP-C analogue of formula (I) in admixture with lipids and phospholipids.

11. A synthetic surfactant according to claim 9, in which the mixture lipids/phospholipids comprises DPPG, PG, PA.

12. A synthetic surfactant according to claims 10-11, further comprising SP-B or an active derivative thereof or a polymyxin.

13. A synthetic surfactant according to claims 10-12, in form of solution, dispersion, suspension, dry powder.

14. Use of SP-C analogues of claims 1-7 for the preparation of a synthetic surfactant to be used in all cases of surfactant deficiencies.

15. Use of a polymyxin, preferably polymyxin B for the preparation of an artificial surfactant according to claims 10-13, for the treatment of all cases of surfactant deficiencies or dysfunction, related pulmonary diseases such as pneumonia, bronchitis, asthma, meconium aspiration syndrome and also other diseases such as serous otitis media (glue ear).

16. Use according to claims 14 and 15, in which the surfactant deficiency is respiratory distress syndrome.

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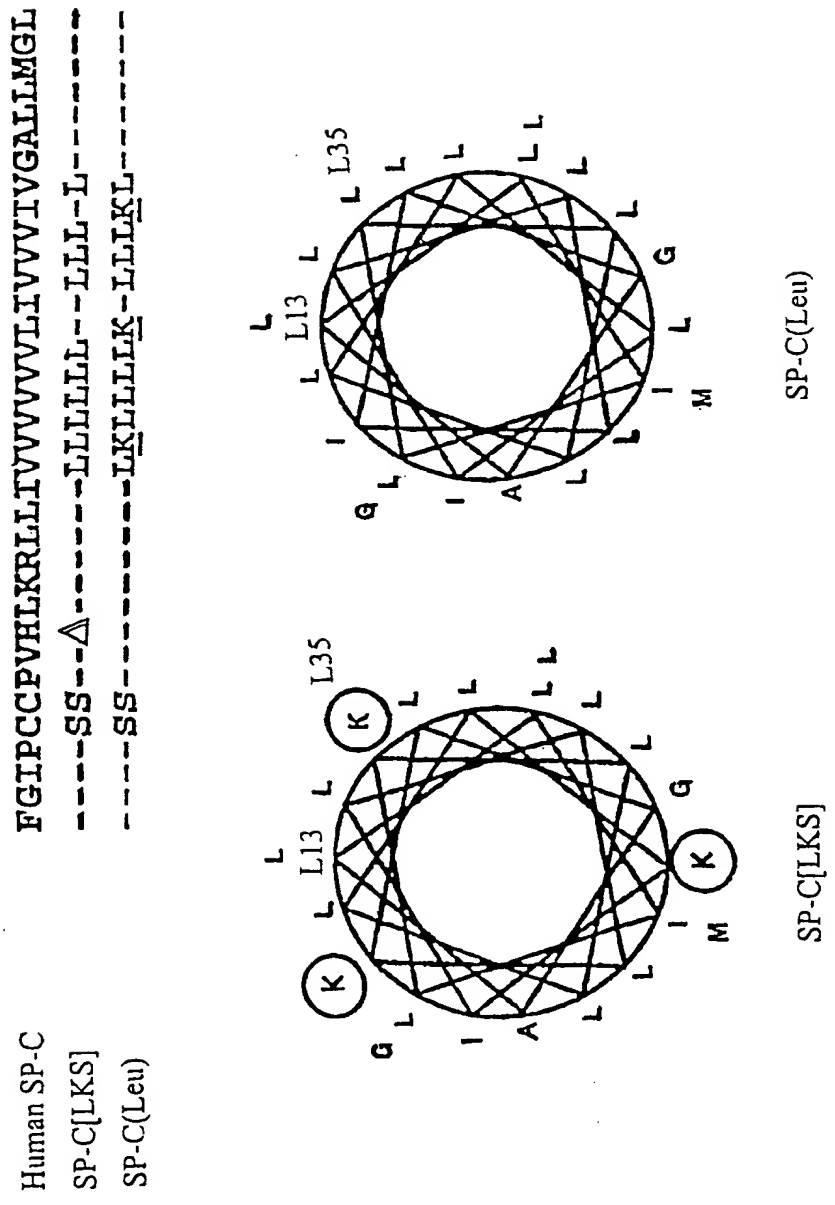
FIGURE 1


Human SP-C FGIPCCPVHLKRLLITVVVVVLIVVVIVGALLMGL
SP-C[LKS] ----SS--Δ------LLLLLL--LLL-L--------
SP-C(Leu) ----SS----------LKLLLLK-LLLKL--------
L13
L35
SP-C[LKS]
SP-C(Leu)

THE FOLLOWING IS THE ENGLISH TRANSLATION OF THE ANNEXES TO THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT : AMENDED SHEETS (Pages 23, 24, 25 and Figure 1).

TENT COOPERATION TR[redacted]Y

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB 527 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/01044	09/02/2000	12/02/1999
International Patent Classification (IPC) or national classification and IPC C07K14/785		
Applicant CHIESI FARMACEUTICI S.P.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/07/2000	22.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01044

I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-22	as originally filed			

Claims, No.:

1-16	as received on	23/02/2001	with letter of	23/02/2001

Drawings, sheets:

2/3,3/3	as originally filed			
1/3	as received on	23/02/2001	with letter of	23/02/2001

Sequence listing part of the description, pages:

1/3-3/3, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence

listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13, 16
	No:	Claims	14, 15
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations

see separat sh et

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

I. Basis

The documents mentioned in the present written opinion / International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

II. Priority

The present claims have been changed in comparison to the content of the priority document. Claim 14 has been broadened since not limited to pulmonary surfactant deficiencies any more. Claim 15 still encompasses this broadened definition. Hence, priority is not acknowledged for these claims.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

- Novelty (Art.33(2) PCT)

The amended set of claims is no longer anticipated by D1-D5.

- Inventive Step (Art.33(3) PCT)

Regarding claims 14 and 15 only. Due to priority situation, D1 is considered relevant to assessment of inventive step of these claims. Since D1 teaches the surfactants of the present invention, it is obvious to employ these to treat surfactant deficiencies of any kind (i.e. not only pulmonary deficiencies which are entitled to priority in these claims). Hence, it is considered obvious in view of D1 to treat otitis media with the surfactants of D1.

Regarding claims 1-13 and 16, It would appear that the specific SP-C(LKS) surfacant depicted in Fig.1 is inventive. There is no teaching in the prior art to space Lycine residues within a primarily Leucine sequence to achieve efficient alpha-helix formation but low aggregation. Prior art merely suggests that double-cysteine needs to be changed to reduce problem.

The claims extend beyond the specific clearly inventive exemplified peptide and

cover replacement of some of the neutral amino acid residues with bulky or polar residues (applicant originally showed that when 3 are replaced which are relatively evenly spaced around the helical circumference a positive effect is achieved). Applicant has now provided data demonstrating that introduction of 1 bulky or polar residue is sufficient. Hence, the number of residues replaced as above is no longer considered problematic. In view of the above, inventive step can now be acknowledged for claims 1-13 and 16.

- **Industrial Applicability (Art.33(4) PCT)**

The present claims appear to have industrial applicability.

VII. **Certain Defects**

The description presently contains a passage which does not acceptably define the invention and in fact gives a false impression of its scope. The passage is found on p.5, l.13-22. Viewing said passage, the definition of an amino acid as neutral would normally only include the polar neutral amino acids N, Q, S, T and Y, yet since these are not found in SP-C it is not clear what to replace. If the definition is taken to include nonpolar amino acids then G, A, V, L, I, P, F, M, W and C are included and these are present both within the alpha-helical core and the flanking sequences. Replacement of these residues in these sequences could lead to a poorly functioning peptide which may also not be protected from aggregation.

CLAIMS

1. SP-C analogues having general formula (I), according to one-letter amino acid code:

$$F_eG_fIPZZPVHLKR(X_aB)(X_bB)_n(X_cB)_mX_dGALLMGL \quad (I)$$

wherein:

X is an amino acid selected from the group consisting of I, L, Nle (norleucine);

B is an amino acid selected from the group consisting of K, W, F, Y, Ornithine;

Z is S and can be optionally linked via ester or thio-ester bonds with acyl group containing 12-22 carbon atoms.

a is an integer from 1 to 19;

b is an integer from 1 to 19;

c is an integer from 1 to 21;

d is an integer from 0 to 20;

e is 0 or 1;

f is 0 or 1;

n is 0 or 1;

m is 0 or 1,

with the following conditions:

- $n + m > 0$;
- $f \geq e$;
- $(X_aB)(X_bB)_n(X_cB)_mX_d$ is a sequence having a maximum of 22 amino acids, preferably from 10 to 22 amino acids.

2. SP-C analogues according to claim 1, having formula (Ia):

(Ia) $FGIPSSPVHLKRX_4BX_4BX_4BXGALLMGL$

3. SP-C analogues according to claim 1, having formula (Ib):

(Ib) FGIPSSPVHLKRX$_5$BX$_5$BX$_4$GALLMGL

4. SP-C analogues according to claim 1, having formula (Ic)

(Ic) FGIPSSPVHLKRX$_4$BX$_{11}$GALLMGL

5. SP-C analogues according to claim 1, having formula (Id)

(Id) FGIPSSPVHLKRX$_8$BX$_7$GALLMGL

6. SP-C analogues according to claim 1, having formula (Ie)

(Ie) FGIPSSPVHLKRX$_{11}$BX$_4$GALLMGL

7. SP-C analogues according to claims 1-6, in which Ser residues are acylated preferably with palmitoyl groups.

8. SP-C analogues according to claims 1-7, in which B is Lysine or Phenylalanine and X is Leucine, Isoleucine or Norleucine.

9. SP-C analogues according to claim 8, selected from the group consisting of:

SP-C (LKS) FGIPSSPVHLKRLLILKLLLLKILLLKLGALLMGL

SP-C (LKS)$_1$ FGIPSSPVHLKRLLILLKLLLLIKLLILGALLMGL

SP-C (LKS)$_2$ FGIPSSPVHLKRLLILKLLLLLILLLILGALLMGL

SP-C (LKS)$_3$ FGIPSSPVHLKRLLILLLLLKLILLLILGALLMGL

SP-C (LKS)$_4$ FGIPSSPVHLKRLLILLLLLLLIKLLILGALLMGL

SP-C (LFS) FGIPSSPVHLKRLLILFLLLLFILLLFLGALLMGL

10. A synthetic surfactant comprising at least one SP-C analogue of formula (I) in admixture with lipids and phospholipids.

11. A synthetic surfactant according to claim 10, in which the mixture lipids/phospholipids comprises DPPG, PG, PA.

12. A synthetic surfactant according to claims 10-11, further comprising SP-B or an active derivative thereof or a polymyxin.

13. A synthetic surfactant according to claims 10-12, in form of solution, dispersion, suspension, dry powder.

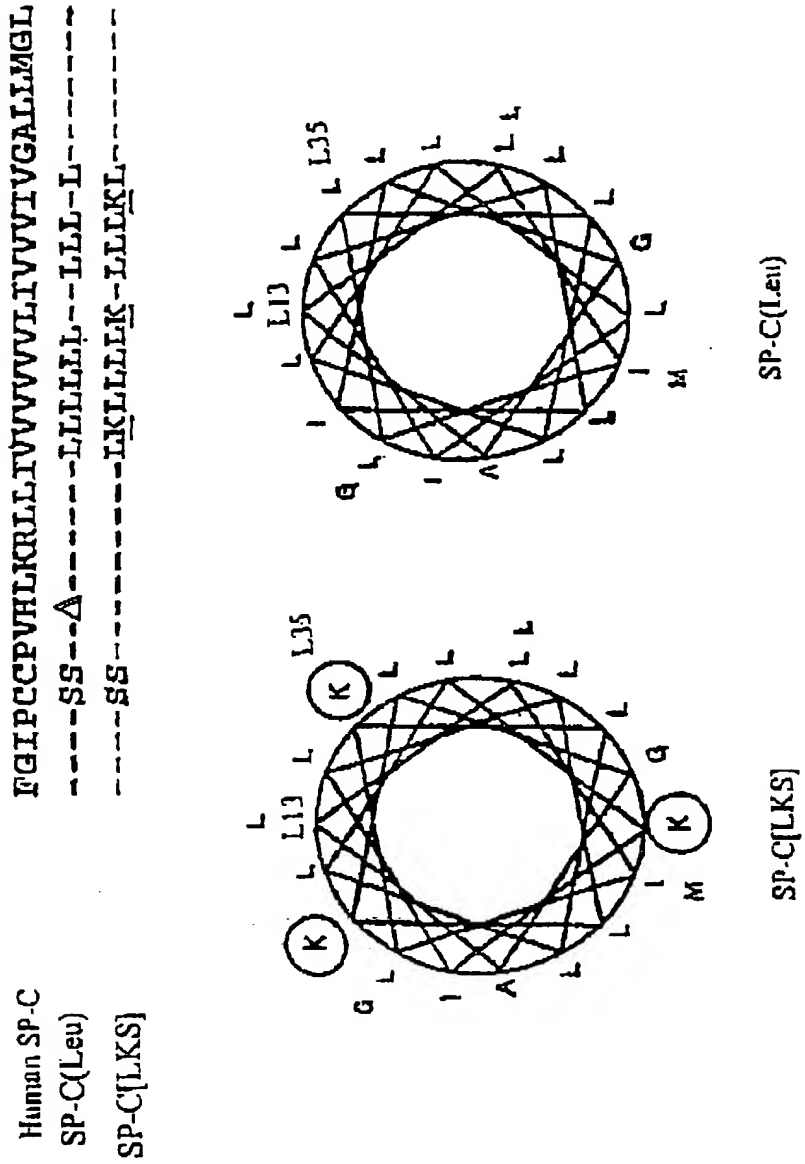
14. Use of SP-C analogues of claims 1-7 for the preparation of a synthetic

surfactant to be used in all cases of surfactant deficiencies.

15. Use of a polymyxin, preferably polymyxin B for the preparation of an artificial surfactant according to claims 10-13, for the treatment of all cases of surfactant deficiencies or dysfunction, or of serous otitis media (glue ear).

16. Use according to claims 14 and 15, in which the surfactant deficiency is respiratory distress syndrome.

FIGURE 1


FGIPCCPVHLKRLLIVVVVVVLIVVVIVGALLMGL
----SS--Δ------LLLLLL--LLL-L--------
----SS----------LKLLLLK-LLLKL--------
Human SP-C
SP-C(Leu)
SP-C[LKS]
L13
L35
SP-C(Leu)
SP-C[LKS]